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14. ABSTRACT <p>The overall goal of this project was to evaluate the processes involved in the attenuation of hexavalent chromium (Cr(VI)) contamination in harbor sediments. Specifically our objectives were to 1) evaluate the mechanisms of Cr(VI) reduction in harbor sediments; 2) evaluate whether bacteria can couple their growth on organic matter to Cr(VI) reduction and if so, whether there is a hierarchy in the use of electron acceptors by these bacteria; and 3) to determine the effects of Cr(VI) on metal- and sulfate-reducing activities and consequential precipitation of Cr.</p> <p>Batch culture and harbor sediment mesocosm experiments examining the effect of Cr(VI) on microbial diversity and processes were performed. The results indicate that Cr(VI) reduction occurs in surficial harbor sediments as a result of microbial activities, primarily those of facultative anaerobic metal-reducing bacteria. As a result, Cr accumulates in the surface sediments. Bacteria capable of using Cr(VI) as an electron acceptor for oxidation of organic matter in the absence of oxygen were isolated and characterized. Cr(VI), even at moderately low levels, dramatically impacts the microbial communities found in sediments.</p>							
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Biological Controls on the Precipitation of Chromium in Harbor Sediments

Bradley M. Tebo

Marine Biology Research Division, Scripps Institution of Oceanography

9500 Gilman Drive

La Jolla, CA 92093-0202

phone (858) 534-5470 fax (858) 534-7313 email btebo@ucsd.edu

Anna Y. Obraztsova (Associate Investigator)

Marine Biology Research Division, Scripps Institution of Oceanography

9500 Gilman Drive

La Jolla, CA 92093-0202

phone (858) 534-0638 fax (858) 534-7313 email annao@ucsd.edu

Award Number: N00014-99-1-0107

LONG-TERM GOALS

Our long term goal is to understand the mechanisms controlling the cycling of metals in the marine environment, more specifically the role of microbial activities in the precipitation of metals and mineral formation. It is important to be able to distinguish biotic from abiotic processes and to determine the relative contribution of the different mechanisms to metal cycling and the microbial populations involved.

OBJECTIVES

The overall goal of this project was to evaluate the processes involved in the attenuation of chromium (Cr) contamination in harbor sites. Specifically our objectives were to 1) evaluate the mechanisms of Cr(VI) reduction in harbor sediments; 2) evaluate whether bacteria can couple their growth on organic matter to Cr(VI) reduction and if so, whether there is a hierarchy in the use of electron acceptors by these bacteria; and 3) to determine the effects of Cr(VI) on metal- and sulfate-reducing activities and consequential precipitation of Cr.

APPROACH

1) We hypothesized that Cr(VI) can be reduced by sulfate-reducing and metal-reducing bacteria by two pathways, a) indirectly through chemical precipitation via the metabolic products HS^- and Fe^{2+} , and b) direct use of Cr(VI) as an electron acceptor. We also hypothesized that the relative importance of sulfate-reducing and metal-reducing bacteria for Cr precipitation would be different depending on the levels of Cr(VI). To demonstrate that sulfate-reducing and metal-reducing bacteria reduce Cr(VI) we used consortia and pure cultures obtained from sediments and measured the disappearance of Cr(VI) or precipitation of solid phase Cr (Cr(III)) with time in defined media in the presence and absence of SO_4^{2-} and Fe(III). Bacterial growth, the metabolic products HS^- and $Fe(II)$, and the effect of different concentrations of Cr(VI) on the relative rates (i.e., kinetics) of Cr(VI) reduction by sulfate-reducing and metal-reducing bacterial cultures were measured. This work was primarily carried out by the specialist, Dr. Anna Obraztsova and graduate student Ms. Y. Meriah Arias, a graduate student working on this project but supported by a NIH fellowship.

2) We hypothesized that the potential energy obtained from the utilization of Cr(VI) as an electron acceptor can partially outweigh the toxic effects of the metal on these bacteria and that the selection among potentially competing electron acceptors is concentration dependent. Since recent evidence suggested that at least some bacteria appear to grow at the expense of Cr(VI) reduction, we tried to elucidate the hierarchy among Cr(VI), Fe(III), and/or SO_4^{2-} with pure cultures by comparing the rates of metal reduction and bacterial growth in experiments with various ratios of Cr(VI) and Fe(III) or SO_4^{2-} in a defined medium. Dr. Obraztsova worked on this aspect of the project.

3) The activities of metal- and sulfate-reducing microorganisms are involved in metal reduction/detoxification processes and are important factors controlling the fate of metals in polluted areas. We hypothesized that metals may affect the diversity and therefore the relative activity of metal- and sulfate-reducing microbial populations. Laboratory mesocosms were set up with a range of added amounts of Cr to evaluate whether the activities of SRB or MRB dominate under different conditions. The effect of Cr(VI) in the overlying water column on bacterial diversity, profiles of redox sensitive species (O_2 , H_2S , and Fe(II)) and sulfate reduction rates was evaluated. Bacterial diversity was examined by denaturing gradient gel electrophoresis (DGGE) and the chemistry was measured using microelectrodes and analysis of solid phase and pore water species in the sediment. The microbiological and molecular biological work was primarily carried out by graduate student Ms. Y. Meriah Arias. The microelectrode work was carried out by a graduate student in the lab, Ms. Karen Murray, and the chemistry work was done by a visiting post doctoral researcher from Mexico, Carlos Green-Ruiz.

WORK COMPLETED

Two mesocosm experiments were set up and run over one year in aquaria with roughly equal proportion of sediments and overlying water. Experiments were conducted with one set of aquaria serving as controls (no added Cr(VI)) and 2 sets in which different levels of Cr(VI) were maintained at approximately constant concentration in the overlying water. At the end of the experiments the sediments were extensively cored and analyzed for chemistry and microbial diversity. We performed voltammetric microelectrode measurements to determine which environmental factors, O_2 , H_2S , or Fe(II), exerted the most significant influence on chromium distribution and mobility. We established various stable anaerobic enrichments from the mesocosm experiments and performed DGGE experiments to evaluate the similarities or differences of the microbial populations exposed to various Cr(VI) concentrations. We have also performed a series of experiments with the isolate for better understanding of microbial physiology/behavior throughout Cr(VI) stress conditions.

RESULTS

With the goal of understanding the role of microorganisms in Cr(VI) reduction/detoxification process and in order to develop a realistic view of natural attenuation as a feasible clean-up strategy, we performed pure culture and mesocosm experiments. Previous experiments with enrichment and pure cultures have provided us with clues as to which organisms might be responsible for detoxification of hexavalent chromium (CrVI). The results from the first mesocosm experiment provided us with basic information about the alterations of bacterial communities under chromate exposure as well as the localization and distribution of chromium concentrations in aquaria sediments.

The second mesocosm study was designed for six 10-gallon (38L) aquaria containing approximately 12 cm of sediment taken from the Marine Facilities Center in San Diego Bay at Point Loma, California. Sea water (20L) from Scripps Institution of Oceanography Pier was used to overlay the sediment. To simulate modest wave action, the water column was stirred with paddles attached to a motor rotating at approximately 60 rpms. Duplicate aquaria with 0.0 mM, 0.05mM and 0.25mM Cr(VI) simulating the control, low, and high contaminant levels Cr(VI) were maintained in the overlying water column for a period of 6 months at 14-16° C. The Cr(VI) concentration was monitored using the diphenyl carbazide colorimetric technique. Stratification occurred and was visible through the sides of the clear aquaria. Each Cr(VI) condition (control, low, and high) had a distinct stratification pattern and was reproducible between duplicate aquaria. A distinct orange layer in these sediments indicated that the sediment is iron-rich. Solid state microelectrodes were inserted into the sediments and moved in millimeter scale intervals with a micromanipulator while voltammetry was used to measure chemical profiles at two different times during the experiment. The results are presented in Table 1.

Table 1. Presence of chemical species in mesocosms

Mesocosm	Oxygen				Iron (II)		Iron (III) Complexes		Manganese (II)		Unknown 1		Unknown 2	
	4/12/01	5/10/01	4/12/01	5/10/01	4/12/01	5/10/01	4/12/01	5/10/01	4/12/01	5/10/01	4/12/01	5/10/01	4/12/01	5/10/01
High A	-	-	-	+	-	+	-	-	-	-	-	-	+	-
High B	-	-	-	-	+	+	+	-	+	+	+	+	-	-
Low A	-	-	-	+	+	+	-	-	+	+	+	+	+	-
Low B	-	-	+	-	+	+	+	+	+	-	-	-	-	+
Control A	+	+	-	+	-	+	-	-	-	-	-	-	+	-
Control B	+	+	-	-	-	-	+	-	-	-	-	-	-	+

Note: sulfide was not seen in any profiles

The redox profiles in duplicate aquaria showed significant differences in chemical species present and the shape of the profiles. Oxygen was not detected in the Cr-enriched tanks, and one of the possible explanations is the inhibition of benthic photosynthetic organisms. The absence of sulfide in any of the tanks is somewhat surprising, although the presence of large amounts of iron (distinct orange layer) could prevent the accumulation of free sulfide in these sediments.

We have also analyzed vertical profiles for the Cr, Mn and Fe concentrations in sediment cores. Each core was sliced by 0.5 cm sections and the section was oven dried (60 °C), ground, and homogenized for chemical analysis. The metal concentrations were measured with a Perkin-Elmer ICP-EOS in a 5% nitric acid solution (50 ml) after digesting 0.25 g of sediment with 0.25 g of lithium tetraborate and 0.5 g of lithium metaborate at 1100 °C. Organic carbon was also determined. Typical vertical profiles of Cr distribution in the sediment cores vs. centimeters intervals are shown in figure 2. The results show that Cr accumulates in the upper horizons of sediments.

In parallel with the chemical analyses, we examined the presence of metal-reducing and sulfate-reducing microorganisms in chromium contaminated and non-contaminated aquaria from the same samples. We inoculated sediment samples into a defined medium with a variety of carbon sources: formate, acetate, propionate + butyrate, or lactate with Cr(VI), Fe(III) or sulfate as electron acceptors in order to score chromium, iron or sulfate-reducing bacteria. We incubated inoculations in an anaerobic chamber using micro-titer plates and have results for iron and- sulfate-reducing bacteria.

There were no significant differences in the number of iron and sulfate-reducing bacteria from the superficial sediment layer in the control and the low Cr aquaria.

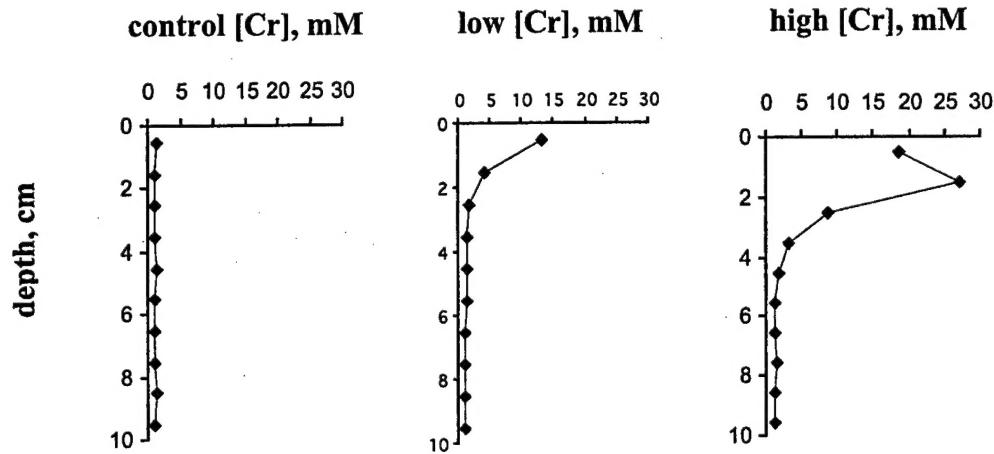


Figure 1. Typical profiles of chromium distributions in the core sediments. Total Cr concentration was measured vs depth of core sediments. Cr accumulation/immobilization was found in the upper sediment horizons in the low and high Cr aquaria relative to control aquaria.

The number of bacteria from all metabolic groups considerably decreased in the high Cr aquaria. An investigation of the microbial population by DGGE also revealed considerable differences in the control and Cr-enriched tanks. All these results are in agreement with our hypothesis that the main biogeochemical scenario for Cr(VI) reduction/removal occurs in the narrow oxic/anoxic zone of a polluted environment and facultative anaerobic bacteria, not sulfate-reducing bacteria, play an important role in this process.

Pantoea agglomerans strain SP1, a facultative anaerobic bacterium, can grow anaerobically with the dissimilatory reduction of a variety of electron acceptors, including Fe(III), Mn(IV), and Cr(VI), but not sulfate. We recently found the additional capacity of this organism to grow via the disproportionation of elemental sulfur to sulfate and sulfide, a process which has previously only been reported in strictly anaerobic members of the δ -Proteobacteria. The sulfide scavengers, Fe(III), Mn(IV), and for the first time, Cr(VI), were found to enhance growth coupled to S^0 -disproportionation. This organism may play a role in the attenuation of Cr(VI) pollution. We found that when Cr(VI) was added to SP1 culture containing elemental sulfur alone, S^0 disproportionation occurred, accompanied by bacterial growth and reduction of Cr(VI). Relative to the control, the S^0 -disproportionating culture reduced Cr(VI) more rapidly and showed Cr(VI) reduction after 6 days (Figure 2A). Sulfate accumulated concurrently with Cr(VI) reduction and reached a concentration about 3-fold higher than with cultures grown with S^0 alone (Figure 2B). These results suggest that Cr(VI) was reduced by HS^- in according the following reaction: $CrO_4^{2-} + 3HS^- + 13H^+ + 4H_2O \rightarrow 8Cr(OH)_3 + 3SO_4$. Cr(VI) reduction and sulfur disproportionation occurred also along with ferric iron and manganese oxide. Our

results suggest that sulfur disproportionation may provide an effective mechanism for the reductive detoxification of Cr(VI) in anoxic environments, particularly those in which the availability of utilizable organic matter (i.e., electron donors) is limited.

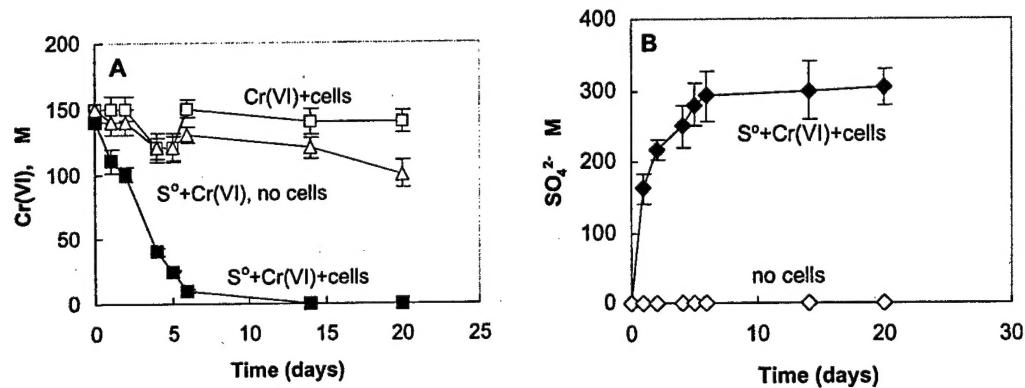


Figure 2. Cr(VI) reduction (A) and sulfate formation (B) by *SP1* during sulfur disproportionation process over time with S^0 and Cr(VI) only. The results are means and SDs from duplicate cultures. A: Bacteria were cultivated with Cr(VI) only and Cr(VI) and S^0 . Reduction of Cr(VI) was only observed in the presence of sulfur. B: Sulfate production confirms sulfur disproportionation and coincided with Cr(VI) reduction.

IMPACT/APPLICATIONS

The overall goal of this project was to address the problem of heavy metal contamination in Navy facilities, specifically chromium (Cr) in harbor sites. Traditional methods for treating contaminated waters, soils, and sediments such as dredging are often expensive, laborious, and may have adverse environmental impacts. The proposed research will help elucidate the complex interplay between direct and indirect effects of microbial processes on Cr precipitation in marine sediments and improve our understanding of some key factors that affect Cr mobility. Ultimately, this information may lead to new strategies for controlling or remediating metal pollution in marine sediments. Specifically, we expect that our data will provide the scientific basis for predicting the extent and pathways for the natural attenuation of Cr in harbor sediments.

TRANSITIONS

None at present.

RELATED PROJECTS

We have performed experiments to examine the mechanisms of Cr(VI) reduction by *Shewanella putrefaciens* strain MR-4, a ubiquitous metal and-sulfur-reducing marine bacterium. Recently, we showed that MR-4 can grow and respire with chromate alone and reduce Cr(VI) in the presence of other electron acceptors. These findings are very important suggesting this strain is useful organism to elucidate the biochemistry of Cr(VI) reduction. We are currently investigating protein(s) expression in these bacteria when exposed to range of concentrations of Cr(VI).

PUBLICATIONS

1. Francis, C. A., Obraztsova, A.Y., and Tebo B.M. Dissimilatory Metal Reduction by the Facultative Anaerobe *Pantoea agglomerans* SP1. 2000. *Applied and Environmental Microbiology* 66: 543-548.
2. Obraztsova A.Y., Francis, C.A., and Tebo B.M. Sulfur Disproportionation by the Facultative Anaerobe *Pantoea agglomerans* SP1 as a Mechanism for Chromium(VI) Reduction. 2002. *Geomicrobiology Journal* 19:121-132.
3. Arias, Y.M. Natural Attenuation of Cr(VI) by Bacteria in Harbor Sediments. 2002. Ph.D. thesis, University of California San Diego, 176pp.
4. Arias Y Meriah; Tebo Bradley M. Cr(VI) Reduction by Sulfidogenic and Nonsulfidogenic Microbial Consortia. 2003. *Applied & Environmental Microbiology* 69: 1847-1853.
5. Arias Y. M., Obraztsova A., Tebo B. M., Green-Ruiz C. Natural Attenuation of Cr(VI) Contamination in Laboratory Mesocosms. 2003. *Geomicrobiology Journal* 20: 389-401.
6. Arias, Y.M., A.Y. Obraztsova, C. Green-Ruiz, and B.M. Tebo. Elucidation of bacterial diversity under hexavalent chromium exposure in laboratory mesocosms (in preparation).
7. Green-Ruiz, C., Y.M. Arias, A.Y. Obraztsova, K.J. Murray, M. Griffith, and B.M. Tebo. Laboratory mesocosm response to Cr(VI) addition: Effects on sediment chemistry (in preparation).